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ORIGINAL ARTICLE

A Putative Pathogen-resistant Regulatory Pathway between MicroRNAs and Candidate Target Genes in Maize

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Abstract MicroRNAs (miRNAs) are a family of small non-coding RNAs that, in most cases, negatively regulate gene expression at the post-transcriptional level. Plant miRNAs have been implicated in developmental processes and adaptation to environmental stress including biotic and abiotic stresses. Here, we report a comprehensive analysis of miRNAs and associated target genes under banded leaf and sheath blight (BLSB) stress caused by *R. solani* in maize. Eight differentially expressed miRNAs were randomly selected from deep sequencing results and validated by qRT-PCR together with their putative target genes, most of which are transcription factors as well as metabolic genes involved in auxin signaling. The results revealed that majorities of the analyzed miRNAs show an inverse correlation with their corresponding predicted target genes. In addition, a putative regulatory network of miRNAs-mRNAs responsive to *R. solani* was constructed. This study provides insight into the regulatory functions of miRNAs, thereby expanding our knowledge of the molecular mechanisms of pathogen resistance.

Keywords: Banded leaf and sheath blight (BLSB), Maize, microRNA, *R. solani,* TFs (transcription factors)

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Introduction

Plants have evolved an immune system to recognize and respond to pathogen attack via many gene regulatory mechanisms operating at transcriptional, post-transcriptional and post-translational levels. MicroRNAs (miRNAs) are a class of small RNAs (21-24 nt) that regulate the expression of target genes at the post-transcriptional level and play an important regulatory role in various biological processes in plants. Furthermore, expression pattern analyses of plant miRNAs by high-throughput profiling or fine-scale quantification have revealed that numerous miRNAs are expressed in a tissue- or stage-specific manner or are induced by external stimuli (Reinhart et al. 2002; Kidner and Martienssen 2004; Sunkar et al. 2007; Yao et al. 2007; Hsieh et al. 2009). Lu et al. (2007) identified and cloned from the stem xylem of loblolly pine (*Pinus taeda*) 26 miRNAs that respond to the fusiform rust pathogen (an endemic rust fungus, *Cronartium quercuum f. sp. fusiforme*), and 82 plant disease-related transcripts were predicted to be responsive to miRNA-guided regulation in the development of fusiform rust galls (Lu et al. 2007). Using wheat leaves infected with the common strain of powdery mildew (*Erysiphe graminis* f. *sp. tritici*), Xin et al. (2010) found 24 microRNAs to be responsive to powdery mildew infection (Xin et al. 2010). However, many microRNA and their putative target genes that are involved in maize responsiveness to *R. solani* have not been identified, and the molecular mechanism of *R. solani*

Table 1. The candidate miRNA selected form differential expression level by deep sequencing

miRNA ID	CK_expressed	Treat_expressed	CK-std	Treat_std	Log2 (Treat/Ck)
Zma-miR159a	4677	1887	515.6092	230.4327	-1.309489006
Zma-miR160a	30	6	3.3073	0.7327	-2.321928095
Zma-miR172b	42520	20201	4687.5565	2466.8632	-1.073714885
Zma-miR393b	120	68	13.2292	8.3039	-0.819427754
Zma-miR394a	83	5	9.1502	0.6106	-4.053111336
Zma-miR166l	15	5	1.6537	0.6106	-1.584962501
Zma-miR396a	29	18	3.1971	2.1981	-0.509949146
Zma-miRn10	472	219	22.8204	10.0135	-1.10785599

tolerance in maize thus remains unknown.

BLSB (banded leaf and sheath blight), which is caused by *Rhizoctonia solani Kühn*, is one of the most important diseases of maize (*Zea mays*. L) worldwide. Severe yield losses can result from the rapid development and large-scale spread of the disease under optimal environmental conditions (Sharma and Saxena 2002). Furthermore, *R. solani Kühn* is a common soil-borne pathogen with a great diversity of host plants and can attack previously resistant cultivars. Moreover, the capacity of fungal spores to travel long distances makes controlling BLSB rather difficult (Zhang et al. 2006). In a previous study, we identified "R15" as an inbred line with a good general combining ability (GCA), good agronomic characteristics and a high level of resistance to BLSB as well as the highly susceptible inbred Ye478 line (Aiguo et al. 2003). Moreover, differentially expressed genes were identified in R15 during *Rhizoctonia Solani Kühn* infection by suppression subtractive hybridization and compared to Ye478 (Zhang et al. 2012). Digital gene expression profiling (DGE) (Gao et al. 2014) and differential expressed protein were also reported as induced by *Rhizoctonia solani Kühn* in different resistant maize inbred lines (Xing et al. 2011), as were the functional effects of different defense enzymes on banded leaf and sheath blight in maize (Li et al. 2009). However, many genes involved in maize resistance to BLSB have not been isolated, and the molecular mechanism of *R. solani* tolerance in maize is yet to be elucidated. It is reported that the microRNA-mediated regulation of gene expression is involved in the pathological development of wheat (Xin et al. 2010), pine (Lu et al. 2007), and tomato (Gu et al. 2010) diseases. In our previous study, R15 was employed to identify a set of candidate microRNAs associated with BLSB resistance using microRNA deep sequencing technology. We attempted to understand how microRNAs regulate their associated target genes and are responsive to BLSB attack between highly susceptible Ye478 and resistant R15. In our present study, we validated candidate microRNAs selected from deep sequencing and their associated target genes in these two materials. Furthermore, our miRNA-target regulation network will be useful in the study of both the regulation of mRNA by microRNAs and the roles of regulation mechanism in plants.

Results

BLSB Stress Alters miRNA Expression in Maize

Based on our deep sequencing data (data not shown), BLSB significantly altered global microRNA gene expression in the highly resistant maize inbred line R15 when comparing the treated library to the control (CK) (Table 1). To demonstrate the functions of these candidate miRNAs, we analyzed the microRNA expression profiles using qRT-PCR in R15 and the highly susceptible maize inbred line Ye478. We confirmed in vivo the expression of Zma-miR159a, Zma-miR172b, Zma-miR394a, Zma-miRn10 (a novel microRNA), Zma-miR160a, Zma-miR166i, Zma-miR396c and Zma-miR393b in maize leaf sheaths in both samples (Fig. 1). In our study, Zma-miR393b showed a decreasing trend in Ye478, but no significant changes were observed in R15 (Fig. 1A); miR394a expression was also decreased in Ye478 but increased in R15 up to 12 h, after which its expression decreased at 24 h (Fig. 1E). Their target genes belong to the F-box family: Zma-miR393-associated target (F-box) was decreased in both R15 and Ye478 (Fig. 2A), whereas Zma-miR394-associated target (F-box) was increased in R15 after 12 h infection and reached a peak at 24 h but was reduced in Ye478 (Fig. 2E). It is probable that Zma-miR394 negatively regulates F-box-like proteins, thereby participating in the ubiquitin degradation pathway through the auxin signaling cascade in response to BLSB attack. However, the reasons for the varying interplay of F-box-like proteins with Zma-miR393 and Zma-miRNA394 remains to be further studied. MYB transcription factor was increased in R15 compared with Ye478 after BLSB infection (Fig. 2B), though the associated candidate microRNA Zma-miR159a showed a downward trend in these two samples (Fig. 1B). Zma-miR160a showed a decreasing trend in R15 and Ye478 during leaf sheath

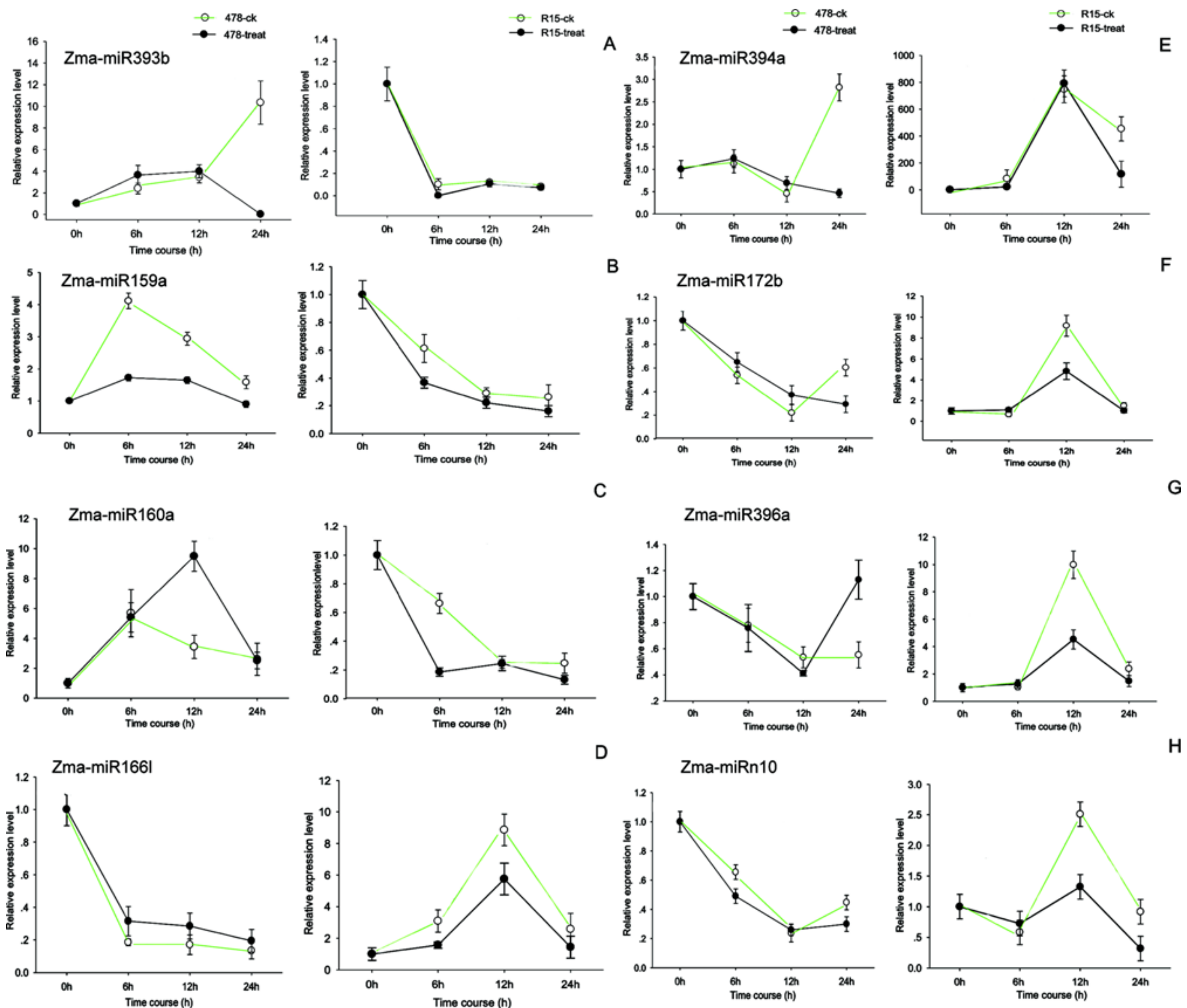

Fig. 1. Validation of candidate microRNAs by qRT-PCR. *R. solani* CK samples correspond to mock-inoculated plants, and treated samples correspond to inoculated plants. From left to right, Ye478 and R15, respectively; the white dot represents CK, and the black dot represents different treatments. From A to H, the expression levels of Zma-miR393b, Zma-miR159a, Zma-miR160a, Zma-miR166i, Zma-miR394a, Zma-miR172b, Zma-miR396a and Zma-miRn10, respectively. Error bars show the standard error calculated from three biological replicates. Stars indicate the significant ($P < 0.05$) difference identified by an uncorrected Fisher LSD test in multiple comparisons after two-way ANOVA.

development under BLSB attack (Fig. 1C). The target gene ARF was increased at 6 h after infection and then decreased at 12 h in R15, in contrast to the expression observed in Ye478 (Fig. 2C). Zma-miR166i showed a downward trend in R15 but an upward trend in susceptible Ye478 (Fig. 1D), and its putative target gene bZIP was negatively regulated in both samples (Fig. 2D). Zma-miR172b was down-regulated in resistant maize inbred line R15 yet up-regulated in Ye478 compared with CK (Fig. 1F). However, the target gene AP2-ERF showed no obvious alteration in R15 but was down-regulated in Ye478 (Fig. 2F). With regard to the Zma-miR396 family, Zma-miR396a exhibited low expression in R15 but was up-regulated in Ye478 (Fig. 1G). Moreover, we found that GRF reached its peak at 12 h after infection in R15 and at 24 h in Ye478 (Fig. 2G), suggesting that miR396a negatively regulates GRFs and is indirectly involved in the regulation of plant cell division and resistance to BLSB attack. A novel miRNA named Zma-miRn10 was down-regulated in both R15 and Ye478 (Fig. 1H). By comparison of the Zma-miRn10 mature sequence with Zma-miR171 family members Zma-miR171a and Zma-miR171b, it is predicted that Zma-miRn10 might be a new member of the Zma-miR171 family (Fig. 4); the putative target gene GRAS was identical to the expression trends of the miR171 family (Fig. 2H). In conclusion, with the exception of Zma-miR396c, the expression levels of

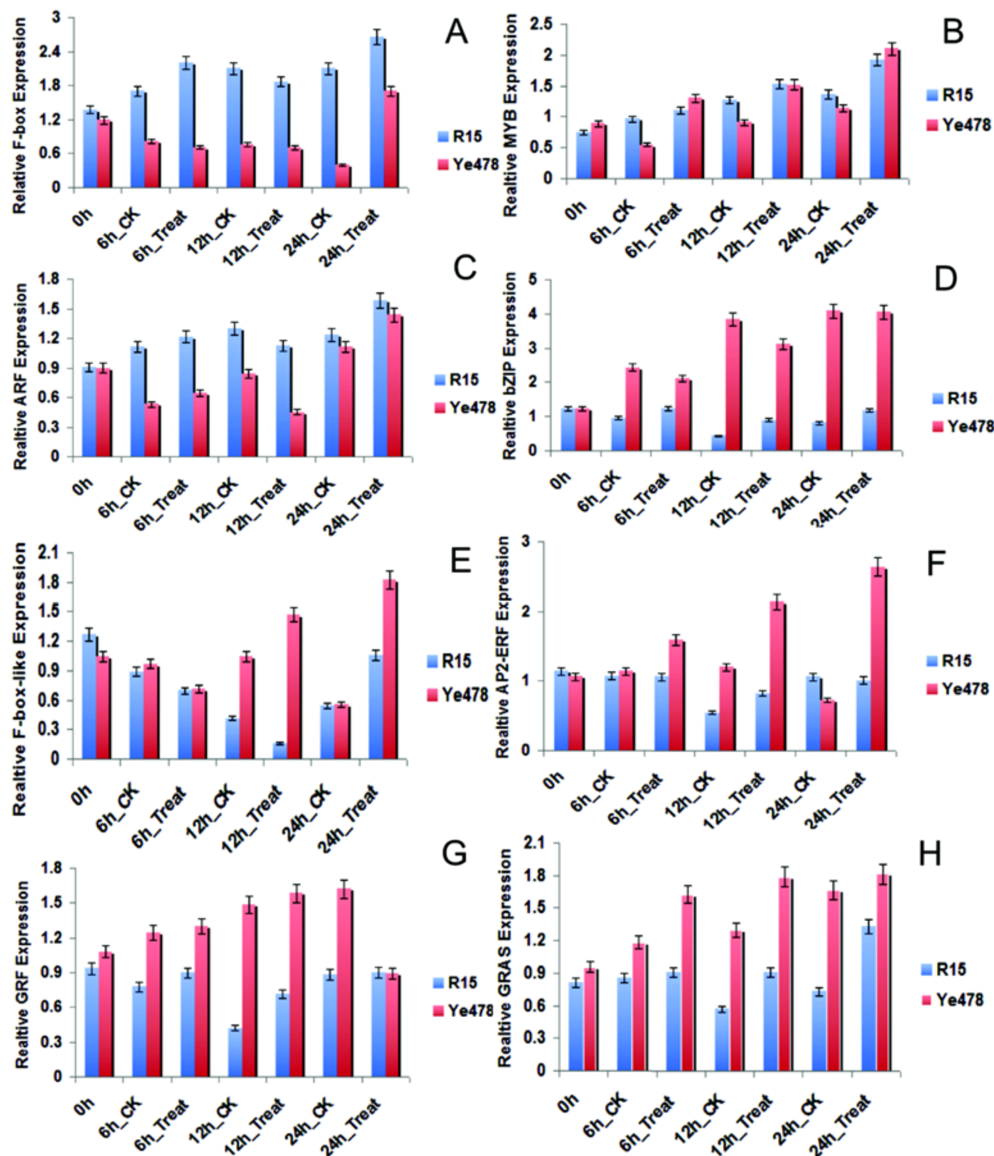

Fig. 2. Validation of the target genes of candidate microRNAs by qRT-PCR. Mock-inoculated plants correspond to CK samples, and inoculated plants correspond to treated samples. The color nodes represent the expression of fold change. From A to H, the expression levels of F-box, MYB, ARF, bZIP, F-box like protein, AP2-ERF, GRF and GRAS, respectively. Error bars show the standard error calculated from three biological replicates. Stars indicate the significant ($P < 0.05$) difference identified by an uncorrected Fisher LSD test in multiple comparisons after two-way ANOVA.

candidate miRNAs were mostly down-regulated in R15, consistent with the deep sequencing results (data unpublished). It is probable that R15 has developed a strong defense system to resist the invasion of this pathogen, such as a thickening of the cell walls, which might participate in both primary and secondary immune responses against pathogen attack.

Prediction and Q-RT-PCR Analysis of Target Genes of Candidate microRNAs

Target genes of the candidate miRNAs were predicted by WMD3 (http://wm d3.weigelworld.org/) and PsRNAtarget (http://plantgrn.noble.org/psRNATarget/) using the ZmB73_ v4a.53 (MGC) database (Table S3). Interestingly, most of the target genes of the candidate microRNAs belong to transcription factors families. Zma-miRNA159 targets the MYB transcription factor family, Zma-miR166 acts on bZIP transcription factors, Zma-miR172 regulates AP2-ERF pathogen factors, Zma-miR396 affects GRFs (growth-regulating factors), and Zma-miRn10 regulates GRAS transcription factors. In addition, some target genes of candidate miRNAs are involved in signal transduction pathways to pathogen attack resistance; for instance, ARF (Auxin Response Factor) genes are regulated by Zma-miR160, and F-box-like protein is regulated by

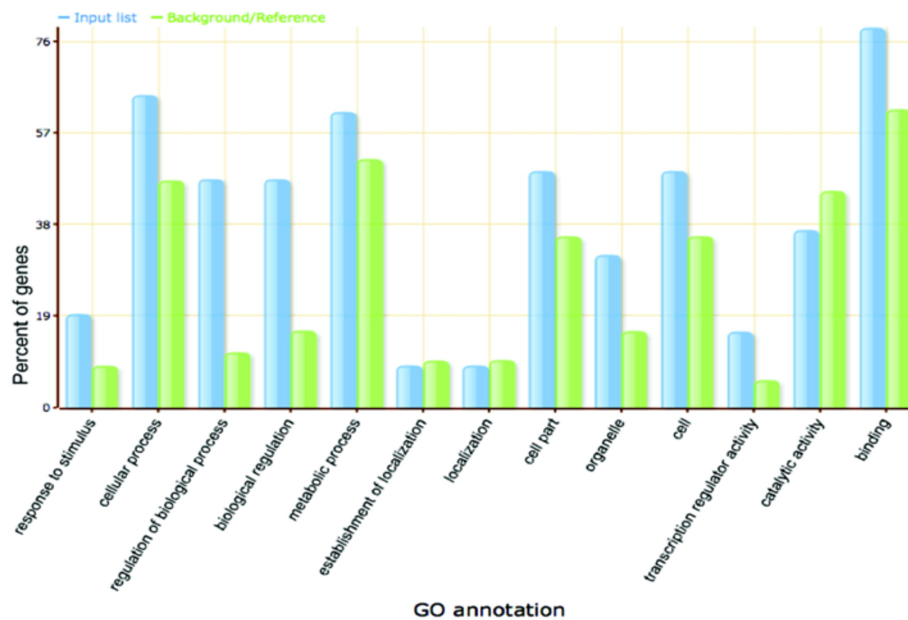

Fig. 3. GO annotation of target genes of candidate microRNAs. The Y-axis is the percentage of target genes mapped by GO terms and represents the abundance of GO term. The X-axis is the definition of GO terms.

Fig. 4. Conservation analysis the mature sequence of Zma-miRn10, Zma-miR171a, and Zma-miR171b.

ma-miR393 and Zma-miR394. Moreover, we found that the expression level of target genes was nearly opposite that of the corresponding microRNAs (Fig. 2). In conclusion, these miRNAs negatively regulate target genes that are involved in transcriptional and post-transcriptional regulatory networks in response to *R. solani* in maize.

GO Annotation and Biological Process Analysis of Target Genes

To gain a better understanding of the functional roles of target genes, a Gene Ontology (GO) analysis, available from B73 RefGen_v2, was preformed. GO terms are commonly used to describe the functions of genes and gene products, thereby facilitating queries among genes from different organisms. Fig. 3 shows the target genes specifically enriched with regard to transcription regulator activity and response to stimulus. The expression levels of the input lists (target genes) are more than 2-fold that of the references.

Discussion

Plants have developed various response mechanisms to resist biotic and abiotic stresses in their natural habitat; they are capable of extensive reprogramming of their transcriptome in a highly dynamic and temporal manner in response to adverse environmental conditions. This response regulation, leading to the adaptive plasticity of plants in highly variable environments, is mainly achieved through a network of various transcription factors (TFs) involved in various plant processes to cope with diverse biotic and abiotic stresses. Moreover, miRNA activities are highly variable at distinct developmental stages, in response to diverse treatments, and in different tissues and are also involved in plant disease resistance pathways via the negative regulation of target gene expression. According to the results from microRNA deep sequencing, we found that most of the miRNAs identified, Zma-miR159a, Zma-miR166i, Zma-miR172b, Zma-miR396a and Zma-miRn10 (novel miRNAs), Zma-miR160a, Zma-miR393b and -miR394a, participate in plant disease resistance by regulating transcription factors (GRAS, GRF, MYB, AP2-ERF, bZIP). In addition, Zma-miR160a, Zma-miR393b and Zma-miR394a are involved in signal transduction pathways, including growth-regulating factors and auxin response factors (ARF), in response to pathogen attack.

Millar AA et al found that miR159a targets transcription factor MYB33, MYB65 and MYC101 of the gibberellin (GA) signal transduction pathway and is activated by a gene homologous to barley GAMYB (Millar and Gubler 2005). It is probable that gibberellin signaling pathway is to a certain extent involved in plant disease resistance pathways. In addition, MYB33 and MYB101 are involved as regulatory

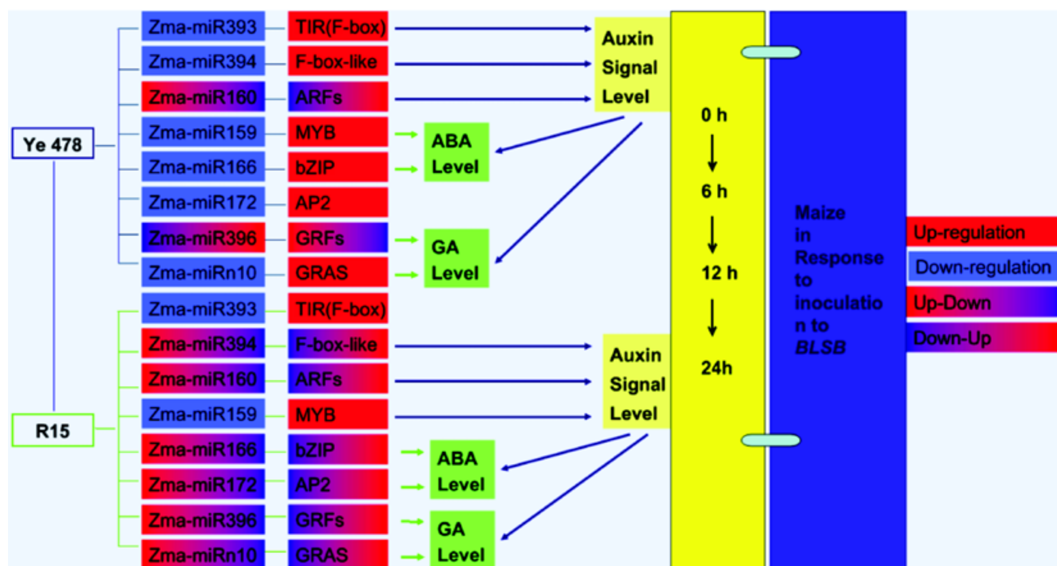

Fig. 5. Putative pathogen resistance regulatory networks in maize responsive to *R. solani.*

factors in ABA signaling in Arabidopsis (Zheng et al. 2006; Reyes and Chua 2007) and indirectly regulate stomatal closure and ion permeability reduction. ABA signaling can also lead to the accumulation of the corpus callosum to improve plant resistance (Lee et al. 2006; Melotto et al. 2006; Zhang et al. 2008), thereby enhancing resistance to disease. Therefore, we speculate that miR159a negatively regulates MYB transcription factors and may be involved in plant disease resistance by activating the GA and ABA signaling pathways to resist BLSB attack. miR396a regulates conserved targets belonging to the GROWTH-REGULATING FACTOR (GRF) family of transcription factors, which are known to control cell proliferation in Arabidopsis leaves (Naqvi et al. 2010). We speculate that the miR396 family is the first barrier to BLSB infection. In recent years, studies have reported that GRAS enhances the plant resistance response as a regulator. It is thought that CIGR1 and CIGR2 (GRAS transcription factors) from rice are induced by N-acetylchitooligosaccharide elicitor perception (Day et al. 2004) and that GRAS genes are important for disease resistance in tomato (*Solanum lycopersicum*) (Mayrose et al. 2006). However, we found that GRAS was down-regulated in R15 and up-regulated in Ye478. Studies have shown that miR160 can enhance the corpus callosum by positively regulating PAMP (Li et al. 2010). We speculate that resistant R15 would quickly reduce pathogen infection via the accumulation of GRAS induced by Zma-miR160a expression. Moreover, ARF proteins can bind to auxin-responsive elements to activate or repress the transcription of primary auxin response genes (Hagen and Guilfoyle 2002). It is reported that auxin can be perceived by F-box proteins, including transport inhibitor response 1 (TIR1) and auxin signaling F-box proteins (AFB1, AFB2, and AFB3) (Dharmasiri et al. 2005; Kepinski and Leyser 2005; Navarro et al. 2006). Zma-miR393 leads to the degradation of the mRNAs of auxin receptors TIR1, AFB2, and AFB3, but the transcriptional repression of AFB1 is microRNA independent and induced by bacterial peptide g22 (Navarro et al. 2006).

Based on the results of the present study, a feedback regulatory network of miRNA-mRNA in maize pathogen response is illustrated in Fig. 5. We divided the identified miRNAs into two categories, with Zma-miR393b, Zma-miR160a and Zma-miR394a being involved in the further regulation of signal transduction pathways by targeting TIR (F-box), ARFs and F-box-like (auxin signaling F-box proteins, AFBs) transcription factors in response to pathogen attack. Zma-miR160a regulate Auxin Response Factors (ARFs) (including ARF10, ARF16 and ARF17 genes), which are involved in responding to stress by binding to auxin-responsive elements. Zma-miR393 and Zma-miR394a target the TIR1-like (F-box) gene and AFBs (auxin signaling F-box like proteins, AFBs), which are both important in the auxin signaling transduction pathway. It is probable that F-box-like transcription factors are directly involved in the ubiquitin degradation pathway; in addition, the auxin level not only activates the GA or ABA signal pathway to resist pathogen infections but also reduces pathogenic virulence as the carbon and nitrogen source that are fully utilized by bacteria to enhance their virulence and their growth obtained from plants. Moreover, auxin is also likely responsible for plant disease resistance by acting as part of the PTI. Regarding the second category, Zma-miR159a, Zma-miR166i, Zma-miR172b, Zma-miR396a and Zma-miRn10, the target genes are predominantly transcription factors, such as MYB, bZIP, AP2-ERF, GRF and GRAS.

MYB domain transcription factors, targeted by Zma-miR159, may be involved in the gibberellin-signaling pathway, with participation in ABA signal transduction in response to pathogen infection. Zma-miR166 regulates bZIP transcription factors, which activate the PR (pathogenesis-related protein) promoter and comprise a diverse group of connected components of plant defense responses against pathogens, thereby associating pathogen defense through the ABA signaling pathway. In addition, Zma-miR172 usually targets AP2-ERF (AP2) transcription factors in the regulation of maize floral organ identity and meristem acquisition, and miR396a regulates GRF transcription factors involved in controlling the proliferation of leaf cells. Zma-miRn10, as a novel microRNA, is predicted to target GRAS transcription factors. During pathogen attack in plants, it is probable that AP2-ERF, GRF and GRAS transcription factors are enhanced to block BLSB infection by regulating the GA signal transduction pathway. In conclusion, this putative miRNA-mRNA feedback network might help to fine-tune gene expression regulation during pathogen attack in plants. To the best of our knowledge, this is the first report on miRNA-mediated gene regulation after pathogen (BLSB) attack in maize. Therefore, the present study may shed light on the research and management of BLSB-related diseases in maize as well as in other plants.

Materials and Methods

Plant Material and Pathogen Infection

High-resistance maize inbred line seedlings of "R15" and high-sensitive maize inbred line seedlings of "Ye478" were treated with 7% hypochlorite solution for 30 min respectively, followed by three washes with sterilized water before being sowed in pots with autoclaved soil. Control plants were maintained under the same conditions. *R. solani* AG1-IA was cultured on potato dextrose agar (PDA) and incubated for three days at 28°C. Agar blocks (0.5-cm squares) were cut and prepared from the outer edge of a 3-d-old culture. Soak barley grains were prepared in water for 24 h and dispensed 40 g in 250 mL conical flask. 2-3 d old pure culture is suspended in distilled sterile water, to make suspension and seed 5 mL of the suspension in each flask and incubate them at 27°C for 10 d. The impregnated grains was later on be used for inoculation. Impregnated barley grains were placed at junction of sheath and leaf during the rainy days when moist condition was prevalent and crop is 30-40 d old. Two to four grains should be inserted between stalk and sheath on second or third internodes level from soil for better inoculation. Subsequently, the leave sheaths were covered with plastic bags to ensure high humidity. The inoculated plants and mock-inoculated plants grew in the same growth chambers in Maize Research Institute of Sichuan Agricultural University. The leaf sheaths were covered with plastic bags to ensure high humidity. The leaf sheaths without any leaves were harvested from each of three maize plants, and the three leaf sheaths were combined to represent one replicate. Three independent replicates were collected for each sample. Finally, inoculated and mock-inoculated bract tissues were collected at 0 h, 6 h, 12 h, and 24 h after 3 d inoculation for the four stages respectively.

Maize Sample Collection and RNA Isolation

All samples from R15 and Ye478 were cleaned and immediately frozen in liquid nitrogen for further biochemical and molecular studies. Small RNA was isolated from each sample using the mirVana™miRNA Isolation Kit (Ambion) following the manufacturer's instructions. Total RNA was isolated from each sample using Trizol Reagent (Invitrogen, Nottingham, UK) according to the manufacturer's instructions. Two small RNA libraries was constructed using RNA extracted and pooled in equal proportions from the leaf sheath of the BLSB resistant maize variety R15 at 0, 6, 12, and 24 h after 3 d inoculation. Briefly, the total RNA were then subjected to 15% denaturing polyacrylamide gel electrophoresis, and the 18-30 nt size range of RNA was isolated from the gel and purified, and subsequently quantified with spectrophotometer (NanoDrop 2000). Next, solexa adapters were sequentially ligated to the 5- and 3-termini of these small RNAs. The gel-purified ligation products were converted to cDNA and amplified by RT-PCR with 18 PCR cycles to produce libraries that were sequenced using a Solexa sequencer at Huada Genomics Institute Co. Ltd, Shenzhen, China, three technical replicates were used for each sample.

Validate the Candidate miRNAs by qRT-PCR

In our study, total microRNA was isolated from leaf sheaths of resistant inbred lines R15 and susceptible inbred lines Ye478 using Plant MicroRNA Extraction Kit (BIOTEKE, Beijing, China), following the manufacturer's instructions. MicroRNA reverse transcription reactions were performed using One Step miRNA 1st cDNA Synthesis Kit (Shenggong, Chengdu, China). The microRNA reverse transcription reactions were incubated in an Eppendorf Mastercycler (Eppendorf North America, Westbury, NY) for 60 min at 37°C, followed by 5 min at 95°C, and then 4°C until further use. The RT-PCR reactions were performed in a 10 μL volume containing 1 μL diluted reverse transcription product, 1×PCR buffer, 0.2 mM dNTPs, 2.0 U EasyTaq DNA polymerase (TransGen Biotech, Beijing, China), and 0.5 μM specific miRNA primer and universal primer (5-TTACCTAGCGTATCGTTGAC-3) on Eppendorf Mastercycler. The PCR reaction conditions used were as follows: 2 min at 95°C, followed by 38 cycles of denaturation for 5 s at 95°C, annealing for 5 s at 55–60°C, extension for 35 s at 70°C, and then finally 4°C. Three replicates were employed for each tested sample and template-free negative controls. Mitochondrial 5S RNA was used as an internal control to normalize all data. The absolute amount of each miRNA was calculated using the $2^{-\Delta\Delta CT}$ method according to the standard curve. The each miRNA level was expressed as $2^{-\Delta\Delta CT}$ mean ± SE. PCR amplification products were confirmed on 1.5% agarose gel. Specific primers used in sets for qRT-PCR were listed in Table S1.

Target Gene Prediction

To predict the associated target genes of their microRNAs, two publicly available prediction website software were selected, including psRNA (http://plantgrn.noble.org/psRNATarget/) and WMD3 (http://wmd3.weigelworld.org/cgi-bin/webapp.cgi?) (Ossowski et al. 2008; Dai and Zhao 2011). A set of rules proposed in earlier reports for miRNA-target prediction were adopted as described below. (1) Allowing one mismatch in the region complementary to nucleotide positions 2–12 of the miRNA, but not at position 10/11. (2) Three additional mismatches between positions 12 and 22 without more than two continuous mismatches. (3) Newly identified maize miRNA sequences were used as custom miRNA sequences. (4) *Zea mays* L. (maize) DFCI Gene Index (ZMGI) Release 19 transcript/genomic library and maize ZmB73 v4a.53 were used as custom maize databases.

Confirmation of Target Genes by qRT-PCR

To monitor all target genes expression of candidate miRNAs, the

sequences of their specific primers were designed by using Primer 5.0 and are listed in Table S2, mitochondrial 18s rRNA was used as an internal control to normalize all data. First-strand cDNA synthesis was performed with 1 μg total RNA from resistant and susceptible inbred lines using a M-MLV reverse transcriptase (Promega). Amplification reactions were performed as 95°C for 10 s and followed by denaturing 95°C for 5 s, annealing 60°C for 10 s, and extension 72°C for 15 s, 49 cycles during the second stage, and 55°C to 95°C to determine dissociation curves of the amplified products. All samples were performed in 3 biological replicates with 3 technical replicates. Statistic analysis of all data was same as the protocol as previously described in detecting candidate miRNAs by qRT-PCR. The each target mRNAs level was expressed as $2^{-\Delta\Delta CT}$ mean ± SEM.

GO Annotation of Putative Target Genes

Target genes annotation was performed by singular enrichment analysis (SEA) through searching the AgriGO database (http://bioinfo.cau.edu.cn/agriGO/)(Du et al. 2010). Enrichment GO terms will be found out after statistical test from pre-calculated background or customized one. The putative physiological functions of sequences were classified according to the Gene Ontology annotation of component function, biological process, and cellular component ontologies.

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Author's Contributions

ZMZ and GTP conceived and designed the research. JG collected samples, generated experimental data, performed the whole data analysis, and drafted the earlier versions of the manuscript. H P, C Z and ML involved the sample collection and partially revised the manuscript. YO S, HJL involved in the sample collection. ML and ZMZ partially revised the manuscript. All authors read, reviewed and approved the final manuscript. All the authors agreed on the contents of the paper and post no conflicting interest.

Supporting Information

Table S1. The primers of candidate miRNAs association with disease-resistance.
Table S2. The primers of TFs (target genes of candidate miRNAs).
Table S3. Target genes of candidate miRNA predicted by WMD3 and PsRNAtarget.

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